Review Article/Artigo de Revisão

Cutaneous leishmaniasis in northeastern Brazil: a critical appraisal of studies conducted in State of Pernambuco

Leishmaniose cutânea no nordeste do Brasil: uma avaliação crítica dos estudos realizados no Estado de Pernambuco

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ABSTRACT

American cutaneous leishmaniasis (ACL) is a complex disease with clinical and epidemiological features that may vary from region to region. In fact, at least seven different *Leishmania* species, including *Leishmania* (*Viannia*) *braziliensis*, *Leishmania* (*Viannia*) *guyanensis*, *Leishmania* (*Viannia*) *lainsoni*, *Leishmania* (*Viannia*) *naiffi*, *Leishmania* (*Viannia*) *shawi*, *Leishmania* (*Viannia*) *lindenbergi*, and *Leishmania* (*Leishmania*) *amazonensis*, have been implicated in the etiology of ACL in Brazil, and numerous phlebotomine sandfly species of the genus *Lutzomyia* have been regarded as putative or proven vectors. Because ACL is a focal disease, understanding the disease dynamics at the local level is essential for the implementation of more effective control measures. The present paper is a narrative review about the ACL epidemiology in Pernambuco, northeastern Brazil. Furthermore, the need for more effective diagnosis, treatment, control and prevention strategies for the affected populations is highlighted. This paper will provide researchers with a critical appraisal of ACL in Pernambuco. Hopefully, it will also be helpful for public health authorities to improve current control strategies against ACL at the state and country levels.

Keywords: Cutaneous leishmaniasis. *Leishmania braziliensis.* Reservoirs. Insect vectors. Diagnosis. Epidemiology.

RESUMO

A leishmaniose cutânea americana (LCA) é uma doença complexa com características clínicas e epidemiológicas que podem variar de região para região. De fato, pelo menos, sete diferentes espécies de *Leishmania*, incluindo *Leishmania* (*Viannia*) *braziliensis*, *Leishmania* (*Viannia*) *guyanensis*, *Leishmania* (*Viannia*) *lainsoni*, *Leishmania* (*Viannia*) *naiffi*, *Leishmania* (*Viannia*) *shawi*, *Leishmania* (*Viannia*) *lindenbergi* e *Leishmania* (*Leishmania*) *amazonensis*, têm sido incriminadas na etiologia da LCA no Brasil, e numerosas espécies de flebotomíneos do gênero *Lutzomyia,* foram considerados vetores suspeitos ou comprovados. Devido ao seu caráter focal, a compreensão da dinâmica da LCA a nível local é imprescindível para a implementação de medidas de controle eficazes. Este trabalho consiste de uma revisão narrativa sobre a epidemiologia da LCA em Pernambuco, nordeste do Brasil. Além disso, se enfatiza a necessidade de maior efetividade no diagnóstico, tratamento, controle e estratégias de prevenção para as populações afetadas. Este artigo fornecerá aos pesquisadores uma avaliação crítica da LCA em Pernambuco. Espera-se também contribuir com as autoridades de saúde pública no aprimoramento das estratégias atuais de controle da LCA nos níveis estaduais e nacional.

Palavras-chaves: Leishmaniose cutânea. *Leishmania braziliensis.* Reservatórios. Insetos vetores. Diagnóstico. Epidemiologia.

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INTRODUCTION

American cutaneous leishmaniasis (ACL) is a complex disease with clinical and epidemiological features that may vary from region to region[1]. Because ACL is a focal disease, understanding the disease dynamics at the local level is essential for the implementation of more effective control measures.

In Brazil, ACL presents variable epidemiological patterns, which may vary according to the phlebotomine sandfly species involved in transmission, population susceptibility and the level of exposure as well as the diversity and competence of reservoir hosts.

A number of studies on ACL in Pernambuco State, northeastern Brazil, have been performed in the past decades and have contributed enormously to the knowledge of ACL ecoepidemiology at the state and country levels[2-7]. In particular, the importance of small terrestrial mammals in the transmission chain of *Leishmania* (*Viannia*) *braziliensis*[7], the most widespread etiological agent of ACL in Brazil, has been demonstrated.

This paper is a narrative review regarding the ACL epidemiology in Pernambuco. Articles were searched in electronic databases (i.e., Medline, Lilacs and SciELO) using the following search terms: leishmaniasis, mucocutaneous leishmaniasis, tegumentary leishmaniasis, *Leishmania*, *Lutzomyia*, and Pernambuco. Search terms were used alone or in combination and with no language or release date restriction (as of January 2012). Studies investigating the epidemiology, diagnosis, etiology, reservoirs and vectors of ACL were included whereas those exclusively focusing on visceral leishmaniasis were excluded. Additionally, references of retrieved articles were searched for relevant papers that could not have been located in the searched databases. The importance of control and prevention strategies for the affected populations and the need for effective diagnostic tools and treatment procedures are discussed. This paper will provide researchers with a critical appraisal of ACL in Pernambuco. Hopefully, it will also be helpful for public health authorities to improve current control strategies against ACL at the state and country levels.

DIVERSITY OF ETIOLOGICAL AGENTS

Seven species of *Leishmania* have been implicated as ACL agents in Brazil: *Leishmania* (*Viannia*) *braziliensis*, *Leishmania* (*Viannia*) *guyanensis*, *Leishmania* (*Viannia*) *lainsoni*, *Leishmania* (*Viannia*) *naiffi*, *Leishmania* (*Viannia*) *shawi*, *Leishmania* (*Viannia*) *lindenberg*, and *Leishmania* (*Leishmania*) *amazonensis*[8]. However, *L.* (*V.*) *braziliensis* is the main ACL etiological agent in Pernambuco and other Brazilian states[1,4,9-11].

In a study conducted in Amaraji and Cortes (two municipalities from the southern Atlantic Forest region) **(Figure 1)** in 1991, two strains isolated from humans and characterized with multilocus enzyme electrophoresis (MLEE) exhibited an enzymatic profile similar to *L.* (*V.*) *braziliensis stricto sensu*, except for the enzymes isocitrate dehydrogenase-nicotinamide adenine dinucleotide phosphate (IDH-NADP) and phosphoglucomutase (PMG), which were different from the reference strain MHOM/BR/75/M2903. These strains were grouped as a new zymodeme IOC-Z45, a variant of the species[12].

Between 1996 and 2000, in a longitudinal study conducted in Raiz de Dentro, Refrigério and Tranquilidade from Amaraji, 30 strains were isolated from patients presenting predominantly single ulcerated lesions. These strains were placed into serodeme 1 with monoclonal antibodies. Through MLEE, they were all identified as *L.* (*V.*) *braziliensis*, although some of them showed different electrophoretic mobility profiles (electromorphs) for the PGM, IDH-NADP and malic enzyme (ME) and were grouped into four new zymodemes: IOC-Z72, IOC-Z73, IOC-Z74, and IOC-Z75[10].

Another study in three different areas in Pernambuco (Amaraji (southern Atlantic Forest), Paudalho (northern Atlantic Forest region), and Moreno (metropolitan region of Recife) **(Figure 1)** was performed in 2009. Sixty-seven samples isolated from patients with different ACL clinical forms were identified as *L.* (*V.*) *braziliensis* serodeme 1 with monoclonal antibodies. Through MLEE, 10 different zymodemes (IOC-Z26, IOC-Z27, IOC-Z45, IOC-Z72, IOC-Z73, IOC-Z74, IOC-Z75, IOC-Z78, IOC-Z105, and IOC-Z106) were found **(Figure 1)**. Most of these zymodemes were classified[9,10]. It should be noted that this was the first report of *L.* (*V.*) *shawi* in Pernambuco.

In a study performed in 2008 in Mundo Novo, a rural community in São Vicente Férrer, a municipality of the *Agreste* region (scrub region) **(Figure 1)** where visceral leishmaniasis has sporadically been diagnosed in dogs and humans, 20 dogs were found to be positive for *L.* (*V.*) *braziliensis* with polymerase chain reaction (PCR). This finding revealed a high exposure level to *Leishmania* spp. in dogs in this area[11] and suggested that dogs might eventually be infected with *L.* (*V.*) *braziliensis*.

These findings strongly indicate that the transmission cycle complexity and the co-existence of two or more species circulating in the same area may be reflected in the genetic polymorphism of this *Leishmania* population. A recent study suggested that the parasite's genetic diversity may be associated with particular transmission cycles, most likely reflecting the adaptation of different parasite clones to distinct vector species[12].

OCCURRENCE AND DISTRIBUTION OF VECTORS

During the 1940s, studies on phlebotomine sandflies in northeastern Brazil reported the occurrence of *Lutzomyia fischeri*, *Lutzomyia evandroi*, *Lutzomyia squamiventris*, and *Lutzomyia migonei* in the Atlantic Forest region of Pernambuco[13]. Since then, approximately 37 sandfly species have been identified in this state[14].

From 1979-1980, a study was performed in Igarassu (northern Atlantic Forest region), and *Lutzomyia whitmani* was the most abundant species[15]. In another study conducted in Amaraji, one of the major ACL foci in Pernambuco, eight species were identified, but again, *L. whitmani* was predominant in domestic and peridomestic areas. Furthermore, this sandfly was found to be naturally infected by *L.* (*V.*) *braziliensis*[4,16]. Other studies have confirmed that *L. whitmani*

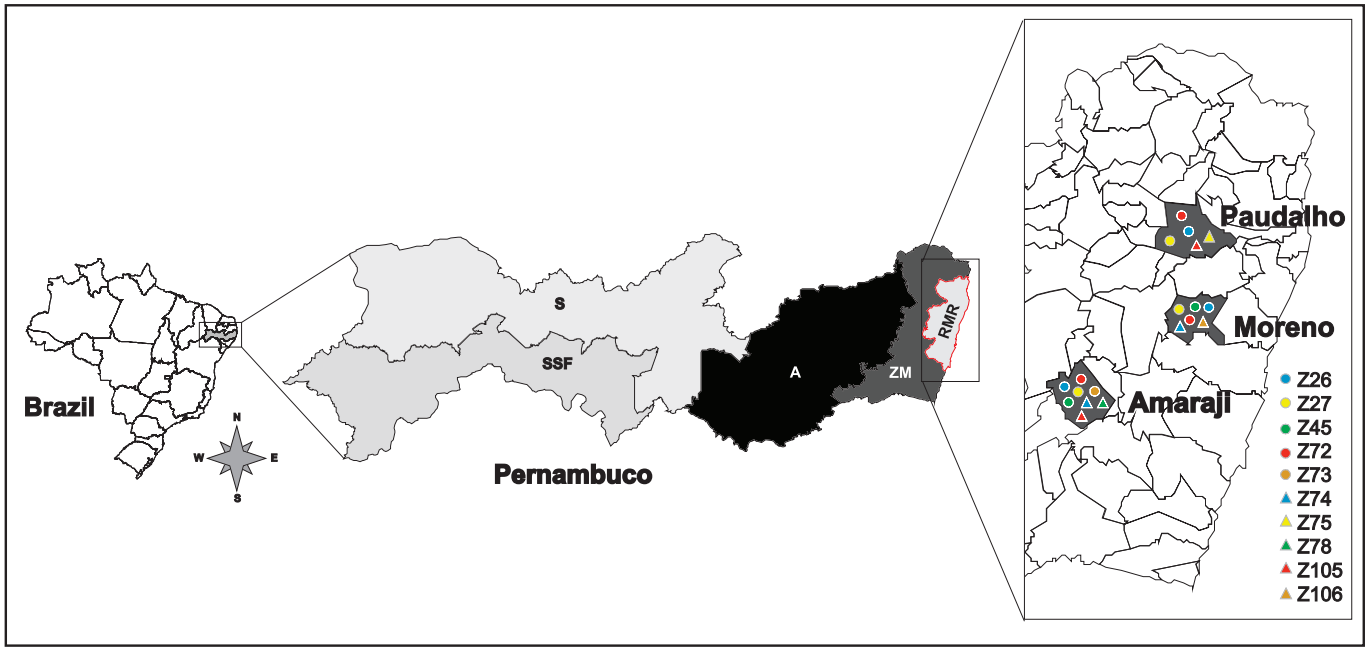

FIGURE 1 - Diversity of *Leishmania* (*Viannia*) *braziliensis* zymodemes in three municipalities (Amaraji, Moreno and Paudalho) of Pernambuco. The geographic regions mentioned (metropolitan region of Recife, Atlantic Forest region, scrub zone and semi-arid region) in the text are also depicted.
RMR: Região Metropolitana de Recife; **ZM:** Zona da Mata; **A:** Agreste; **S:** Sertão; **SSF:** Sertão do São Francisco.

is adapted to the domestic environment and can also be found indoors in Amaraji[16]. Moreover, this species is highly anthropophilic[17], which partly explains the high prevalence of ACL in this area. A more recent investigation in the municipality of Moreno also revealed the predominance of *L. whitmani*[6]. Undoubtedly, *L. whitmani* is the most important vector of *L.* (*V.*) *braziliensis* in Pernambuco.

A recent survey performed in São Vicente Férrer demonstrated that in contrast with Igarassu and Amaraji, *L. migonei* was the predominant species in domestic and peridomestic environments, whereas *Lutzomyia complexa* and *Lutzomyia sordelli* were predominant in forest remnants[18]. Similarly, a study conducted in a military training area in Paudalho reported a high diversity of phlebotomine sandflies, with the predominance of *Lutzomyia complexa* and *Lutzomyia choti*[3,5]. In contrast, in a large study in an Atlantic Forest reserve located in Recife, the most abundant species was *Lutzomyia umbratilis*[19]. This sandfly is a vector of *L.* (*V.*) *guyanensis*, whose presence in Pernambuco is uncertain.

The phlebotomine sandfly fauna of Pernambuco is rich. However, *L. whitmani* is predominant in most of the ACL transmission foci, mainly in domestic and peridomestic areas, whereas *L. complexa* or other species prevail in wild environments[5]. Compared with other northeastern Brazilian states, the phlebotomine sandfly fauna of Pernambuco is more diverse than Alagoas, Ceará, Paraíba, Piauí, Rio Grande do Norte, and Sergipe but not Maranhão and Bahia[13]. The high abundance and widespread distribution of phlebotomine sandfly vectors in Pernambuco indicate that most of the population is exposed to *Leishmania* spp. infection.

WILD AND SYNANTHROPIC RESERVOIR HOSTS

Leishmania (*V.*) *braziliensis* has been detected in several wild and synanthropic rodents of the genera *Akodon*[20], *Proechimys*[21], *Rattus*[2,22], *Oryzomys*[2,23,24], *Rhipidomys*[25], *Nectomys* and *Necromys*[2] and marsupials of the genus *Didelphis*[2,21]. In most cases, the isolates from these animals have neither been properly identified nor characterized; the identification was typically based on morphological characteristics and the behavior of the parasites in culture only.

A pioneering study targeting small mammals as probable *Leishmania* (*V.*) *braziliensis* reservoirs in Pernambuco was conducted in Amaraji, and a total of 407 rodents and 71 marsupials were trapped and analyzed. Remarkably, amastigotes in liver and spleen imprints consistent with *Leishmania* spp. were found in specimens of *Nectomys squamipes* (n=5), *Necromys* (syn. *Bolomys*) *lasiurus* (n=5), and *Rattus rattus*[2]. Another study in the same area presented evidence of *Leishmania* infection in several rodent species. Five *L.* (*V.*) *braziliensis* isolates from *N. lasiurus* and one from *R. rattus* were identified and characterized, and different zymodemes were identified[7]. It is noteworthy that the zymodeme IOC-Z74 was isolated from human patient samples, sentinel hamsters, vectors and both wild and synanthropic rodents, suggesting that this is the predominant zymodeme in the region[7,10]. Considering the difficulties related to the cultivation, isolation and maintenance of *L.* (*V.*) *braziliensis* parasites, these results represented an important achievement[26]. Similarly, a study in São Vicente Férrer also provided evidence of *Leishmania* infection in *N. squamipes*, *R. rattus*, and *Holochilus sciureus*[27] with PCR.

Usually, these animals display no visible lesions consistent with ACL despite being parasitized, suggesting that the *Leishmania* parasites in their primitive form have adapted to these hosts[22]. Therefore, these animals have the potential to act as long-term maintainers of *L.* (*V.*) *braziliensis* in nature[7]. The identification of *N. squamipes*, *N. lasiurus* and *R. rattus* hosting *L.* (*V.*) *braziliensis* represents a major contribution to the knowledge of the epidemiology of ACL in Brazil and allows for a better understanding of the role of these species as reservoirs of this important parasite[7].

Although serological and/or molecular evidence of *Leishmania* (*V.*) *braziliensis* in domestic dogs from ACL endemic areas have been reported[2,11], further research is necessary to ascertain the role of these animals in the ACL transmission chain in Pernambuco.

AMERICAN CUTANEOUS LEISHMANIASIS IN HUMAN POPULATIONS

From a historical perspective, the first ACL cases recorded in Pernambuco date back to the 1930s, when the disease was detected in rural workers from the Atlantic Forest region[28,29].

From 1989 to 1991, 1.604 cases were reported to the Ministério da Saúde (MS), most (64.2%) of which were detected in the Atlantic Forest region[2], particularly in the municipalities of Amaraji and Cortês. In the beginning of the 1990s, an active search for ACL cases in this area identified 127 human cases[2]. Subsequent studies showed a 10-fold increase in ACL over a 10-year period in the region[4]. From an epidemiological standpoint, the infection was more prevalent in adult males (but was also recorded in females) and children, eventually affecting all of the members of the same family[4].

In 1996, an outbreak of ACL occurred in a military training camp (*Campo de Instrução Marechal Newton Cavalcanti* - CIMNC, Paudalho) in a region of somewhat preserved Atlantic Forest, affecting 26 trainee soldiers. An epidemiological investigation in this area detected a 24.1% infection prevalence in that population[3]. From 1996 to 2010, 197 ACL cases were diagnosed among trainee soldiers from CIMNC; an average of 16 cases per year. Therefore, it was concluded that a primary enzootic cycle was established in this area with a somewhat defined fluctuation of cases, with sporadic outbreaks followed by low-occurrence periods[5,30].

In 2010, 25 ACL cases were registered in an outbreak in Igarassu, and an active search detected 49 additional cases in the region[31]. The diagnosis was mostly based on the presence of skin lesions in conjunction with the microscopic detection of amastigotes in skin samples and/or a positive Montenegro skin test. Most patients presented with typical localized lesions. Different age classes (>10 years) and both sexes were affected, although most of the patients were males[31].

Using the Montenegro skin test, a study conducted in Moreno reported a 30% positivity in a population of 481 individuals. The positivity was higher among males and among individuals aged 11 to 30 years. Interestingly, 67% of the positive individuals had no previous ACL history[6].

From 2001 to 2010, 4.855 ACL cases were recorded in Pernambuco, with an average of 485 cases per year[32]. In fact, ACL is endemic in all of the geographical regions of Pernambuco, and outbreaks are sporadically detected in the *Agreste* and Atlantic Forest regions[4]. Although the highest prevalence of ACL is still recorded in the Atlantic Forest region[7], the disease is spreading to other areas, and the incidence is increasing in the entire state. Importantly, most of the affected people live in poor villages in rural areas and are permanently exposed to the disease risk factors, typically for occupational reasons, as observed in other Brazilian regions[33].

AMERICAN CUTANEOUS LEISHMANIASIS DIAGNOSIS

The routine diagnosis of ACL in Pernambuco, particularly in rural areas, is based on clinical and epidemiological criteria. In Recife, the state's capital, both traditional (e.g., cytology) and modern techniques (e.g., PCR) are available in reference hospitals and research centers.

A study performed in 2000 on the antibody response of patients from an endemic ACL area with immunoblotting using antigenic *L.* (*V.*) *braziliensis* fractions identified the 27 and/or 30 kDa soluble antigens, which were considered promising for ACL diagnosis[34]. Immunoblotting was more sensitive (91%) and specific (100%) than the indirect immunofluorescence antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA). In 2001, the same antigenic fractions were employed to analyze the antibody response level in patients before and after treatment[35]. Because the levels of these antigens were decreased approximately twofold in clinically recovered patients, it was concluded that they may be used as markers of healing or parasite persistence in human patients.

The introduction of molecular biology techniques has greatly improved the diagnosis of ACL in Pernambuco and elsewhere in Brazil. Two specific systems for the subgenera *Viannia* and *Leishmania* have been assessed, revealing 95.5% and 88.2% sensitivity, respectively, and 100% specificity for healthy individual samples[36]. Indeed, PCR-based methods are significantly more sensitive than the conventional tests, such as direct examination, histopathological examination and skin sample culture.

Polymerase chain reaction, culture and histopathological examination were also compared in 32 samples from clinically cured ACL patients after chemotherapy. *Leishmania* (*Viannia*) sp. Deoxyribonucleic acid (DNA) was detected in 93.7% of the patients, and three strains were identified through culture. In the histopathological examination, no parasite was found. However, fibroblastic changes were present in all of the cases, with an inflammatory focus observed in four cases, suggesting the persistence of parasites in these patients after chemotherapy[37]. This result was confirmed by the parasite isolation from (n=3) individuals' scars 5 years after clinical cure[37].

In a study involving 19 patients with lesions compatible with ACL from different localities of Pernambuco, 89% of them were positive with the Montenegro skin test, 79% with IFAT, 58% with skin cytology and 75% with PCR[38]. These results clearly illustrate the importance of combining different diagnostic techniques to improve the detection level.

FINAL COMMENTS AND CONCLUSIONS

Many ecoepidemiological aspects of ACL in Pernambuco have been characterized in recent years as an important contribution to the understanding of the infection prevalence and disease expression. Moreover, the characterization of vectors and reservoir hosts involved in the transmission and maintenance of *L.* (*V.*) *braziliensis* in this region has also been assessed.

Leishmania (*V.*) *braziliensis* is the main etiological agent of ACL in Pernambuco, where 10 or more variants circulate. Phlebotomine sandfly vectors are widely distributed over the state's territory, with the predominance of *L. whitmani* in domestic and peridomestic areas and *L. complexa* in forest remnants in most ACL foci. Concerning *L.* (*V.*) *braziliensis* reservoirs, *N. squamipes*, *N. lasiurus* and *R. rattus* have been suggested as possible parasite maintainers in natural transmission cycles, but further transmission studies are needed to better understand their role. For instance, *R. rattus*, originally from Europe, established as synanthropic animals throughout Brazil and now it can be found both in human dwellings and in the fields. On the other hand, from an evolutionary viewpoint, its relationship with *L.* (*V.*) *braziliensis* is recent and it is unknown to what extent this imported rodent has adapted to this native parasite and vice versa.

The Atlantic Forest region is responsible for more than 60% of all of the ACL cases reported in Pernambuco, which emphasizes the need to establish and strengthen preventive and control measures against the disease in this region. Nonetheless, it is also important to promote continuous education for physicians and the capacity of public health professionals working in newly detected ACL foci to speed diagnostic and treatment procedures in these areas.

The diversity of *Leishmania* species or variants found in vertebrate hosts and vectors reflects the complexity of ACL in Pernambuco, which makes the design and implementation of control programs for this endemic disease a challenge for public health authorities. It is expected that this paper will provide public health authorities with essential information on ACL in this Brazilian state, which may be helpful for designing more effective control programs. Current control strategies have not been sufficient to stop or even reduce the disease burden.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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